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Evidence That the Apolipoprotein E–Genotype Effects on Lipid Levels Can Change with Age in Males: A Longitudinal Analysis

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Summary

We previously reported that change, with age, in plasma levels of total cholesterol (TC) and LDL cholesterol (LDL-C) differed between apolipoprotein E (APOE) genotypes ε3ε3 and ε3ε4, in a sample of 77 older, unrelated males. By use of a larger sample from that cohort, followed longitudinally during 1969–87, the change in TC and in LDL-C, between the ε3ε3 and ε3ε4 APOE genotypes, over three exams, was reanalyzed. Additionally, the change in triglycerides (TG) and in HDL-cholesterol (HDL-C), between the ε3ε3 and ε3ε4 APOE genotypes—as well as the differences between the ε3ε3 and ε3ε2 genotypes, for TC, LDL-C, TG, and HDL-C—were contrasted over the three exams. At exam 1 TG was higher in the ε3ε4 group than in the ε3ε3 group (mean age 48 years), and at exams 2 and exam 3 (mean ages 58 and 63 years, respectively) it was similar (P = .009 for the exam-by-genotype–interaction effect in the repeated-measures analysis). A similar trend was seen for TC (P = .03), yet previously detected LDL-C effects were not apparent (P = .46). Those with the ε3ε2 genotype had higher TG and lower LDL-C and TC at each exam than were seen in those with the ε3ε3 genotype, although the differences in the values were not always statistically significant. Differences in TC, LDL-C, and TG, between the ε3ε2-genotype and ε3ε3-genotype groups, did not significantly change over the three exams. HDL-C levels were relatively stable over the exams; however, the exam-by-genotype interaction was significant for the ε3ε2 genotype versus the ε3ε3 genotype (P = .02). The ε4 allele effects on TG and TC changed between longitudinal exams and may be age dependent. Changes, with age, in the effect of the ε3ε4 genotype on lipids may impact the risk of developing atherosclerotic disease.

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Introduction

Apolipoprotein E (apoE) is a component of very-low-density lipoprotein (VLDL) and HDL particles and is a ligand for lipoprotein receptors. The apoE-gene locus on chromosome 19 (APOE) has three common alleles—ε2, ε3, and ε4—with relative frequencies .08, .77, and .15, respectively; the protein products of these alleles differ by a single amino acid (Zannis et al. 1981; Cumming and Robertson 1982; Sing and Davignon 1985). The apoE polymorphism has been estimated to be responsible for 4%–8% of the variation in age- and sex-adjusted total cholesterol (TC) and LDL cholesterol (LDL-C) levels in the general Caucasian population (Sing and Davignon 1985; Boerwinkle and Sing 1987) and may contribute to risk of coronary artery disease (CAD; see reviews by Davignon et al. 1988; Mahley 1988). The ε4 allele has been reported to have an average effect of increasing TC by 5–8 mg/dl and increasing LDL-C by 7 mg/dl. The ε2 allele has been reported to have an average effect of decreasing TC and LDL-C by 11-14 mg/dl (Sing and Davignon 1985). Meta-analysis suggests that, relative to the ε3 allele, both the ε2 allele and the ε4 allele may be associated with increases in plasma triglyceride (TG) levels (Dallongeville et al. 1992). The ε4 allele was found to be associated with earlier age of myocardial infarction (MI), in studies of MI survivors (Cumming and Robertson 1984; Lenzen et al. 1986) with angiographic evidence of CAD (Kuusi et al. 1989) and with clinical ischemic disease (Wilson et al. 1994). APOE-genotype distribution has been found to differ between MI survivors and controls (Cumming and Robertson 1984). However, other studies have failed to find an association with presence or severity of CAD (Menzel et al. 1983; Reardon et al. 1985). ApoE molecules derived from the three alleles vary in their in vitro receptor-binding ability (Mahley 1983), in their catabolic rates (Gregg et al. 1986; Demant et al. 1991), and in the plasma concentration of apoE protein present in vivo (Utermann 1985). The apoE polymorphism effects on lipids and lipoproteins may be mediated by these differences.

Davignon et al. (1987) did not find apoE polymor-

phism effects on TC and LDL-C in octogenarians. At the same time, they found a decrease in the frequency of the ε4 allele in octogenarians, which has been documented in other aging populations (Cauley et al. 1993; Schachter et al. 1994; Jarvik et al. 1995). They suggested that differential survival, based on lipid differences, accounted for both the loss of ε4 alleles and the equalization of TC and LDL-C. An alternative hypothesis—that the equalization of lipids was an aging effect and that the loss of ε4 alleles in the older samples was likely due to the subsequently found association of the ε4 allele with Alzheimer disease (Corder et al. 1993; Yu et al. 1994; Jarvik et al. 1995)—was supported by evidence of a longitudinal decline in the TC and LDL-C of men with the ε3ε4 genotype at age 48–63 years, compared with no difference in TC and LDL-C by the age of 48–63 years in men with the ε3ε3 genotype without evidence of loss of ε4 alleles (Jarvik et al. 1994). Although a statistically significant result was found, the sample size—77 unrelated individuals—was small. The goal of the present study is to reevaluate the results of Jarvik et al. in a larger sample drawn from the same population and to extend it to examine both the longitudinal effect of the ε2 allele and the effects of all alleles on longitudinal change in TG and HDL cholesterol (HDL-C).

Subjects and Methods

Subjects

The study subjects were participants in the National Heart, Lung, and Blood Institute (NHLBI) Twin Study (Feinleib et al. 1977; Selby et al. 1991), composed of Caucasian male twin pairs born during 1917–27, all of whom had served in the U.S. military. Of 560 DZ pairs originally solicited, 260 participated in clinical examinations during 1969–73, and 183 pairs returned for a second examination during 1980–81. A third examination of 129 of the original 260 pairs was conducted during 1986–87. Of the 260 DZ pairs initially examined, 58 individuals in 55 pairs died between the first and third examinations (Reed et al. 1991, 1993). Of the 505 MZ pairs solicited, 254 pairs participated in the first exam, 179 pairs returned for the second exam, and 138 pairs returned for the third exam. Of the original 253 MZ pairs, 38 individuals from 36 pairs died prior to the third exam (Selby et al. 1991). Thus, 96 (9.3%) of the original 1,026 subjects died before the third exam.

APOE genotypes were determined for all individuals with available samples from the third exam. Genotypes were available for 590 men: 122 DZ pairs, 124 MZ pairs, and 41 DZ and 57 MZ singletons whose twins were not available. The subset of the NHLBI twin sample on which genotypes were available had age and lipid distributions typical of the total group (table 1). These distributions in the total NHLBI sample have been shown to be typical of the age-specific population lipid distributions (Jarvik et al. 1994). Because of small sample sizes, the ε4ε4, ε2ε2, and ε2ε4 genotypes were not included in the contrasts. Additionally, 43 individuals who either were under treatment for non–insulin-dependent diabetes or were treated with lipid-lowering medications at any of the three examinations were excluded, leaving 436 individuals available for analysis.

Table 1

Mean Age and Lipid Levels in the Entire NHLBI Twin Sample and in Those with APOE Genotypes Who are Considered in This Study

	All NHLBI Twins	All Those with APOE Genotypes
Exam 1:		
Age (years)	47.9 (n = 1,028)	47.8 (n = 590)
TC (mg/dl)	220.3 (n = 1,023)	221.0 (n = 587)
LDL-C (mg/dl)	143.6 (n = 1,010)	145.7 (n = 560)
TG (mg/dl)	133.1 (n = 1,017)	132.4 (n = 585)
HDL-C (mg/dl)	45.3 (n = 1,013)	46.03 (n = 560)
Exam 2:		
Age (years)	57.6 (n = 784)	57.7 (n = 548)
TC (mg/dl)	211.6 (n = 785)	213.3 (n = 548)
LDL-C (mg/dl)	135.1 (n = 764)	136.6 (n = 529)
TG (mg/dl)	161.2 (n = 785)	161.8 (n = 548)
HDL-C (mg/dl)	45.6 (n = 784)	46.2 (n = 547)
Exam 3:		
Age (years)	63.2 (n = 622)	63.2 (n = 590)
TC (mg/dl)	220.2 (n = 584)	220.6 (n = 560)
LDL-C (mg/dl)	148.2 (n = 550)	148.2 (n = 528)
TG (mg/dl)	147.3 (n = 584)	148.3 (n = 560)
HDL-C (mg/dl)	45.6 (n = 584)	45.8 (n = 560)

Analyses considered genotyped individuals who attended and had TC measured at all three exams. Unrelated subsamples drawn from the twins assured the independence of observations, which is an assumption of the statistical tests. To allow maximum sample sizes, separate subsamples were drawn for the ε2ε3-versus-ε3ε3 analysis and for the ε3ε4-versus-ε3ε3 analysis (Jarvik et al. 1994). In each case, all singletons with a genotype of interest were included in the unrelated sample. One twin from each MZ pair was randomly chosen. For the DZ pairs, siblings with the rarer ε2ε3 and ε3ε4 genotypes, rather than their ε3ε3 siblings, were preferentially included in the unrelated sample, to increase the sample size of those genotypes. The first subsample constructed included 71 ε3ε4 individuals and 177 ε3ε3 individuals; the second included 32 ε2ε3 individuals and 183 ε3ε3 individuals. For both subsamples, mean ages were 48 years at exam 1, 58 years at exam 2, and 63 years at exam 3. At the third exam, the age range was 59–70 years.

We previously reported (Jarvik et al. 1994) the differences in the TC and LDL-C changes, with age, between ε3ε3 and ε3ε4 genotypes of 77 men from the larger

sample selected, without knowledge of lipid levels or of CAD status, from third-exam participants. The current analyses for the ε3ε4 and ε3ε3 unrelated individuals are reported for subsamples both with and without both those individuals who were considered in the original report and their twin brothers, to evaluate the comparability of the current results and the original results.

Methods

Laboratory methods.—TC and TG were measured enzymatically (Allain et al. 1974). HDL-C was estimated by the method of Warnick et al. (1982). LDL-C was estimated (Friedewald et al. 1972) only if the measured TG (Sampson et al. 1975) level was <400 mg/dl (Warnick et al. 1990). At exam 1 the analyses were done in three labs (Framingham, Indianapolis, and San Francisco [Christian et al. 1976]). The labs exchanged specimens, to maintain standardization. There was no difference in mean, by lab, for TC, LDL-C, or TG. The Framingham lab did have a lower mean for HDL-C (4–5 mg/dl [Christian et al. 1976]). At exam 2 the West Coast lab was at Stanford (Christian et al. 1987). All labs again used the standardized methods. All lipid tests at exam 3 were done at Stanford (Christian et al. 1990). APOE genotyping was performed by use of digestion of PCR amplification products with *Hha*I, as described by Hixson and Vernier (1990).

Statistical analyses.—Preliminary analyses included computation of the relative allele frequencies, by allele counting and χ^2 goodness-of-fit testing, to determine whether the numbers of individuals with each of the six possible genotypes were in proportions consistent with Hardy-Weinberg population expectations. Unrelated individuals were selected for statistical analyses, in order to avoid violation of statistical tests. Statistical tests that include related individuals should be interpreted with caution.

In the unrelated subsets, repeated-measures analysis of variance (ANOVA) was used to test the hypothesis that the change in TC, LDL-C, TG, or HDL-C levels at exams 1, 2, and 3 (categorical measures) differed between the ε3ε3 and the ε3ε4 genotypes and between the ε3ε3 and the ε2ε3 genotypes. The repeated-measures test used the SPSS MANOVA command (SPSS 1991; Sheeber et al. 1996). No assumptions were made with regard to a straight-line pattern over exams. Repeated-measures analysis included a genotype effect and an exam effect, in addition to the testing for an exam-by-genotype–interaction effect, in the prediction of TC, LDL-C, TG, or HDL-C levels. All repeated-measures analyses were repeated on TC, LDL-C, ln-TG, and ln-HDL-C, adjusted for age at intake, by use of the residuals resulting from linear regression. Similarly, those analyses were repeated after adjustment for body-mass index (BMI). Additionally, the HDL-C repeated-measures analysis was repeated after deletion of the samples from Framingham, which had significantly different means at exam 1.

The differences in TC, LDL-C, TG, or HDL-C, between exam 3 and exam 1, were also contrasted between the ε3ε3 and ε3ε4 genotypes and between the ε3ε3 and the ε2ε3 genotypes, by use of two-sample *t*-tests. Within exams, one-way ANOVA was used to test whether genotype groups differed in their mean differences of TC, LDL-C, TG, or HDL-C, within exams 1, 2, and 3. Because of statistically significant skewness in the distribution of TG and HDL-C at one or more exams, TG and HDL-C were ln transformed (ln-TG) for all statistical tests, except for the test of exam 3 minus exam 1 differences. Outliers with TG >500 mg/dl were excluded from statistical tests involving TG: this resulted in the exclusion of two ε3ε3-genotype men from the repeated-measures sample. Although multiple comparisons were made in this study, the major goal was to look for exam-by-genotype interaction, and the variables and test hypotheses are highly correlated. For this reason, no adjustments for multiple comparisons were made. All above-described statistical analyses used the SPSS (1991) statistical package.

The proportion of variance due to variation in APOE genotype was computed as the variance associated with APOE genotype, V_E, divided by the total variance, V_T, where $V_E = \Sigma_j f_j(\hat{\mu}. . - \hat{\mu}_j)^2$ when $j = 1, . . . , 6$ APOE genotypes and when f_j represents the frequency of the jth genotype. Sib-pair linkage methods, using Sibpal 2.6a (Tran et al. 1994), were attempted for the following traits: TC, LDL-C, ln-TG, and ln-HDL-C at each exam and their change between exams 1 and 3. However, a maximum of only 115 DZ pairs for whom both sibs were not excluded were available, and power was insufficient to detect evidence of linkage for any trait.

Results

Preliminary Analyses

The APOE-genotype distribution in the 590 men was as follows: 11 (1.9%) ε2ε2, 50 (8.5%) ε2ε3, 22 (3.7%) ε2ε4, 374 (63.3%) ε3ε3, 121 (20.5%) ε3ε4, and 2 (2.0%) ε4ε4 individuals. For the unrelated group of 344 men (with no exclusions), the relative allele frequencies were as follows: .075 for ε2, .779 for ε3, and .145 for ε4. These relative allele frequencies were similar to those seen in other, nonelderly Caucasian populations (Sing and Davignon 1985). The genotype distribution was not significantly different from Hardy-Weinberg equilibrium proportions ($P = .43$). Both results suggest that significant selection against a genotype is not present in this sample. The lipid levels for all individuals present at all three exams who who were not taking lipid-lowering or antiglycemic oral medications are shown in ta-

ble 2. The proportion of the variance, in TC, LDL-C, and TG, associated with the APOE genotype was estimated by use of randomly drawn unrelated individuals from each exam who were not taking lipid-lowering or antiglycemic oral medications. The range of these estimates was 1.7%–7.4%; they are shown in table 3. The TC and LDL-C estimates are somewhat lower than those in younger, mixed-gender samples available from the literature, which are also shown and referenced in table 3, although no other male-only samples were identified. The estimates for variance associated with TG are higher than those previously reported for younger, mixed-gender samples, even when the total TG variance is not previously adjusted for age and BMI. However, Haviland et al. (1995) have reported, in octogenarian males, a higher proportion of variance due to APOE genotype. The proportion of the variance in unadjusted HDL-C levels that is attributable to APOE genotype was estimated to be 1.1%, 1.8%, and 0.9%, respectively, for exams 1, 2, and 3. Estimates for the proportion of variance due to APOE genotype do not differ markedly in all those for whom data were available at each exam, versus those who came to all three exams. This further suggests that the individuals who came to all three exams and who therefore were useful for the longitudinal analysis are representative of the larger group.

ANOVA to test for differences in TC, LDL-C, ln-TG, and HDL-C between genotypes within each exam was performed on the unrelated subsets adjusted for age at entry. The group means are shown in table 4. At each exam, differences between ε3ε3 and ε3ε4 genotypes were not statistically significant, although the results were marginally significant for ln-TG ($P = .059$) at exam 1 only. Inclusion of related individuals would result in statistical significance ($P = .002$). Differences between the ε2ε3 genotype and the ε3ε3 genotype were present in only TC at exam 2 ($P = .021$) and LDL-C at exam 3 ($P = .031$), although the results were marginally significant for TC at exam 3 ($P = .079$), for LDL-C ($P = .09$) at exams 1 and 2 ($P = .08$), and for ln-HDL-C at exam 2 ($P = .065$). A Fisher Z-test was used to determine whether there were statistically significant differences in the TC-TG, TC–LDL-C, and LDL-C–TG Pearson correlation coefficients between genotypes, considering those unrelated individuals used in the repeated-measures analyses. Only the LDL-C–TG correlation difference between the ε3ε3 ($r = .28$) and ε3ε4 ($r = -.02$) genotypes and the LDL-C–HDL-C correlation difference between the ε3ε3 ($r = .09$) and ε3ε4 ($r = -.20$) genotypes, at exam 1, were statistically significant at the .05 level. The LDL-C–TG correlation was not significantly different at exam 2 ($r = .17$ for ε3ε3 and $r = .07$ for ε3ε4) or at exam 3 ($r = .13$ for ε3ε3 and $r = .021$ for ε3ε4). Nor was the LDL-C–HDL-C correlation ($r = -.07$ for ε3ε3 and $r = .04$ for ε3ε4 at exam 2; and $r = -.06$ for ε3ε3 and $r = .15$ for ε3ε4 for exam 3). The TC–TG correlations did not differ significantly at any exam ($r = .40$, .34, and .25 for ε3ε3 at exams 1, 2, and 3, respectively; and $r = .27$, .30, and .03 for ε3ε4 at exams 1, 2, and 3, respectively). No correlation differences between ε3ε3 and ε2ε3 genotypes were detected at any exam. Power may be limited for some of these contrasts. Change in TC, LDL-C, ln-TG, or ln-HDL-C between exams 1 and 3 was significantly different between the ε3ε3 and ε3ε4 genotypes only for ln-TG ($P = .005$) and TC ($P = .023$); between exams 1 and 3, there was no significant difference between the ε2ε3 and ε3ε3 genotypes, for change in TC, LDL-C, ln-TG, or ln-HDL-C.

Repeated Measures

Unrelated individuals, enriched for either the ε2ε3 or the ε3ε4 genotype, as described in the Methods subsection, were used for the repeated-measures analysis; their mean lipid levels are plotted in figure 1. Repeated-measures ANOVA found significant interaction between exam and ε3ε3 versus ε3ε4 genotype in the prediction of TC ($P = .034$) and ln-TG ($P = .009$) but not in prediction of LDL-C ($P = .47$) or ln-HDL-C ($P = .91$) (table 4). Interaction of TC and ln-TG, between the ε3ε3 and ε3ε4 genotype, indicates that the differences were not consistent over exams; this appears to be due to a difference at exam 1, which is not seen at the later exams. Exclusion of previously studied men and their brothers resulted in a nonsignificant result for exam-by-genotype interaction in TC prediction ($P = .75$), although the interaction term for ln-TG remained highly significant ($P = .008$). There is no apparent reason for differences between the 77 men previously reported and their brothers versus the men from pairs that were not previously reported, nor were there statistically significant differences between those from pairs that were or were not previously studied in any test considered here (data not shown).

For the ε2ε3 genotype and the ε3ε3 genotype, repeated-measures ANOVA found no significant exam-by-genotype interaction in the prediction of TC ($P = .52$), LDL-C ($P = .72$), or ln-TG ($P = .48$). No interaction between ε2ε3 and ε3ε3 genotypes indicates that the differences in mean levels between these groups were consistent over exams. However, significant exam-by-genotype interaction for the ε2ε3-to-ε3ε3 contrast was significant for ln-HDL-C ($P = .019$), apparently because of differences at exam 2 (fig. 2). When the exam 1 samples from the Framingham lab were removed, 26 ε2ε3-genotype and 141 ε3ε3-genotype individuals remained, and the P value dropped to .057, without a change in the trend of the exam means. Adjustment of TC, LDL-C, ln-TG, and ln-HDL-C for in-

Table 2

Mean ± SD TC, TG, LDL-C, and HDL-C Levels Stratified by Genotype, for All Subjects Present at All Three Exams Who Were Not on Lipid-Lowering or Antiglycemic Oral Medications

	TC (mg/dl)			TG (mg/dl)			LDL-C (mg/dl)			HDL-C (mg/dl)		
	Exam 1	Exam 2	Exam 3	Exam 1	Exam 2	Exam 3	Exam 1	Exam 2	Exam 3	Exam 1	Exam 2	Exam 3
ε2ε2	195 ± 32 (n = 10)	188 ± 50 (n = 10)	199 ± 44 (n = 10)	130 ± 71 (n = 10)	151 ± 78 (n = 10)	160 ± 85 (n = 10)	102 ± 35 (n = 9)	101 ± 30 (n = 9)	117 ± 32 (n = 9)	43 ± 7 (n = 10)	49 ± 10 (n = 10)	43 ± 7 (n = 10)
ε2ε3	209 ± 38 (n = 44)	199 ± 35 (n = 44)	204 ± 41 (n = 44)	115 ± 60 (n = 44)	148 ± 57 (n = 44)	140 ± 67 (n = 44)	132 ± 32 (n = 43)	125 ± 31 (n = 43)	131 ± 36 (n = 43)	47 ± 14 (n = 44)	45 ± 15 (n = 44)	45 ± 12 (n = 44)
ε2ε4	215 ± 30 (n = 17)	208 ± 31 (n = 17)	216 ± 39 (n = 17)	132 ± 80 (n = 17)	148 ± 58 (n = 17)	111 ± 58 (n = 17)	139 ± 29 (n = 17)	134 ± 29 (n = 17)	144 ± 38 (n = 17)	43 ± 10 (n = 17)	45 ± 10 (n = 17)	50 ± 15 (n = 17)
ε3ε3	219 ± 38 (n = 294)	214 ± 35 (n = 294)	222 ± 38 (n = 294)	114 ± 71 (n = 292)	148 ± 85 (n = 292)	133 ± 87 (n = 292)	146 ± 35 (n = 275)	138 ± 33 (n = 275)	149 ± 35 (n = 275)	47 ± 13 (n = 288)	48 ± 13 (n = 288)	47 ± 12 (n = 288)
ε3ε4	226 ± 35 (n = 98)	216 ± 34 (n = 98)	221 ± 39 (n = 98)	148 ± 112 (n = 98)	158 ± 122 (n = 98)	147 ± 128 (n = 98)	151 ± 31 (n = 86)	141 ± 30 (n = 86)	154 ± 35 (n = 86)	46 ± 13 (n = 97)	45 ± 11 (n = 97)	46 ± 12 (n = 97)
ε4ε4	241 ± 30 (n = 9)	230 ± 27 (n = 9)	229 ± 36 (n = 9)	172 ± 97 (n = 9)	210 ± 132 (n = 9)	193 ± 201 (n = 9)	166 ± 34 (n = 7)	154 ± 26 (n = 7)	165 ± 39 (n = 7)	46 ± 14 (n = 9)	45 ± 10 (n = 9)	43 ± 12 (n = 9)
Total	219 ± 37 (n = 472)	213 ± 36 (n = 472)	219 ± 39 (n = 472)	123 ± 82 (n = 470)	153 ± 93 (n = 470)	138 ± 98 (n = 470)	145 ± 34 (n = 437)	137 ± 32 (n = 437)	148 ± 36 (n = 437)	47 ± 13 (n = 465)	47 ± 13 (n = 465)	46 ± 12 (n = 465)

Table 3

Proportion of Variance, in Lipid levels, Associated with Variation in APOE Genotype

Source	Sample	Mean Age [Range] (years)	Variance (%)		
			TC	LDL-C	TG
Sing and Davignon (1985)[a]	Ottawa (n = 102; 55 male)	36 [20–59]	6.9	8.3	.9
Boerwinkle et al. (1987)[b]	French (n = 223; 109 male)	40 [25–67]	8.7	...	.0
Boerwinkle and Utermann (1988)[c]	German (n = 563; 419 male)	27 [18–59]	4	...	...
Haviland et al. (1995)[d]	118 Males	86 [80–100]	10.5 [8.4]	11.7 [8.7]	12.1 [8.6]
Present study[e]	Exam 1 (n = 330, 317, 328)	48 [44–52]	2.6 (4.2)	7.4 (8.1)	2.8 (2.6)
Present study[e]	Exam 2 (n = 304, 299, 296)	58 [54–62]	3.3 (3.2)	3.6 (3.8)	4.7 (5.7)
Present study[e]	Exam 3 (n = 312, 296, 312)	63 [59–70]	1.7 (2.0)	4.7 (5.7)	3.6 (3.6)

[a] Data are adjusted for variance due to age, sex, height, and weight.

[b] Data are adjusted for variance due to age, sex, height, weight, and hormone usage.

[c] Data are adjusted for variance due to age and sex.

[d] Estimates are adjusted for variance due to varying concomitants (in square brackets are estimates without prior adjustments for concomitants). TC and LDL-C were adjusted for uric-acid levels; and TG was adjusted for height, weight, glucose level, and uric-acid levels.

[e] Data are for a randomly chosen unrelated group of men with genotypes available from exam 3 who are not taking lipid-lowering or oral hypoglycemic medications. No outliers are excluded. LDL-C is only measured when TG are <400 mg/dl. For this study, $n = x, y, z$ are the sample sizes with TC, LDL-C, and TG measured, respectively. The TC, LDL-C, and TG data in parentheses are for the 284 people who had cholesterol measured at all three exams. The variance estimates from this study are not adjusted for variance associated with age and BMI within each exam. That variance is <2% for TC and LDL-C at all exams but varies by 12%–15% for TG at each exam. Variance estimates from other studies may be computed by methods different from that used for this study.

take age or BMI did not change the conclusions of any of the repeated-measures analyses.

Discussion

This has study resulted in several new findings. There is, from this longitudinal study, evidence of an exam-by-genotype interaction in the prediction of TG; that is, the pattern of change in TG, over exams, as the men became older differed between ε3ε3 and ε3ε4 genotypes, after adjustment for age at entry. For TC, LDL-C, and TG, no exam-by-genotype interactions were detectable for ε3ε3 versus ε3ε2. Such an interaction was detected for HDL-C, although it is a marginal result if, because of the problem with standardization, the samples done at the Framingham lab at exam 1 are dropped from the analysis. The proportion of variance in TC and LDL-C that was due to variance in APOE genotype was lower—and that of TG was higher (table 3)—in this older, male sample than that elsewhere reported in younger samples of mixed gender (Sing and Davignon 1985; Boerwinkle et al. 1987; and Boerwinkle and Utermann 1988). The TC, LDL-C, and TG variance associated with APOE genotype was higher in a sample of healthy octogenarian males (Haviland et al. 1995). After adjustment for age and BMI, the variance associated with APOE genotype is <2% for TC and LDL-C, at all exams, but is 12%–15% for TG at each exam (table 3, footnote). This suggests that in some populations (in this case, older males) the APOE effect on TG is more important than previously recognized and the APOE effect on TC and LDL-C may be less important.

An exam-by-genotype interaction is also suggested when ε3ε3 and ε3ε4 genotypes for TC are considered. For both TC and LDL-C the differences between genotypes are small at exam 1, smaller than those reported elsewhere (Jarvik et al. 1994) and those described in younger samples (Sing and Davignon 1985). Some differences between APOE-genotype means for TC and LDL-C may have already diminished by exam 1 (mean age 48 years) in the population described here. This is also suggested by the lower estimates of variance due to APOE genotype that were seen in this sample versus samples reported elsewhere (table 3), although it is possible that males have less variance due to APOE genotype than do females, although the opposite result was reported in healthy octogenarians (Haviland et al. 1995). Additionally, a difference in TC–TG correlations, between genotypes, which has been reported for younger populations (Boerwinkle et al. 1987), was not detectable at any exam in this sample. The exam-by-genotype interaction for TC may reflect the cholesterol contained in VLDL-C, which is highly correlated with TG, since the LDL-C fraction of TC had no evidence of an interaction. The contrast between the prior result of a significant interaction term for LDL-C and the current result of no evidence of interaction is not explained by differences in the samples. The prior result could represent a type I error—false rejection of the null hypothesis of no interaction. Further longitudinal studies are warranted.

Table 4

Mean ± SD TC, TG, LDL-C, and HDL-C Levels at Each Exam, Stratified by Genotype, in Unrelated Subsample

	TC (mg/dl)			TG (mg/dl)			LDL-C (mg/dl)			HDL-C (mg/dl)		
	Exam 1	Exam 2	Exam 3	Exam 1	Exam 2	Exam 3	Exam 1	Exam 2	Exam 3	Exam 1	Exam 2	Exam 3
ε3ε3	218 ± 36 (*n* = 177)	214 ± 36 (*n* = 177)	223 ± 38 (*n* = 177)	116 ± 68 (*n* = 175)	158 ± 94 (*n* = 177)	141 ± 95 (*n* = 177)	145 ± 34 (*n* = 171)	135 ± 32 (*n* = 174)	150 ± 34 (*n* = 169)	47 ± 13 (*n* = 172)	47 ± 13 (*n* = 177)	46 ± 12 (*n* = 177)
ε3ε4	226 ± 37 (*n* = 71)	217 ± 34 (*n* = 71)	219 ± 39 (*n* = 71)	143 ± 10 4(*n* = 71)	163 ± 96 (*n* = 71)	141 ± 11 9(*n* = 71)	147 ± 36 (*n* = 70)	141 ± 30 (*n* = 69)	153 ± 33 (*n* = 65)	46 ± 13 (*n* = 70)	45 ± 11 (*n* = 71)	45 ± 12 (*n* = 71)
ε3ε3[a]	218 ± 35 (*n* = 129)	214 ± 35 (*n* = 129)	221 ± 35 (*n* = 129)	116 ± 67 (*n* = 127)	152 ± 82 (*n* = 129)	134 ± 68 (*n* = 129)	145 ± 34 (*n* = 125)	137 ± 31 (*n* = 128)	149 ± 35 (*n* = 126)	46 ± 13 (*n* = 126)	47 ± 13 (*n* = 129)	46 ± 11 (*n* = 129)
ε3ε4[a]	220 ± 34 (*n* = 44)	218 ± 33 (*n* = 44)	221 ± 39 (*n* = 44)	126 ± 58 (*n* = 44)	146 ± 71 (*n* = 44)	116 ± 54 (*n* = 44)	149 ± 30 (*n* = 43)	143 ± 31 (*n* = 44)	154 ± 33 (*n* = 43)	45 ± 11 (*n* = 43)	46 ± 11 (*n* = 44)	47 ± 12 (*n* = 44)
ε2ε3	210 ± 41 (*n* = 32)	199 ± 36 (*n* = 32)	210 ± 45 (*n* = 32)	116 ± 63 (*n* = 32)	152 ± 62 (*n* = 32)	147 ± 75 (*n* = 32)	135 ± 33 (*n* = 32)	126 ± 30 (*n* = 32)	135 ± 39 (*n* = 31)	47 ± 10 (*n* = 32)	43 ± 12 (*n* = 32)	46 ± 12 (*n* = 32)
ε3ε3[b]	219 ± 38 (*n* = 183)	215 ± 35 (*n* = 183)	223 ± 37 (*n* = 183)	118 ± 79 (*n* = 181)	156 ± 93 (*n* = 183)	138 ± 90 (*n* = 183)	145 ± 35 (*n* = 177)	137 ± 31 (*n* = 180)	150 ± 33 (*n* = 176)	47 ± 13 (*n* = 178)	48 ± 13 (*n* = 183)	46 ± 12 (*n* = 183)

[a] Data exclude previously reported individuals and their brothers.
[b] Comparison group for ε2ε3.

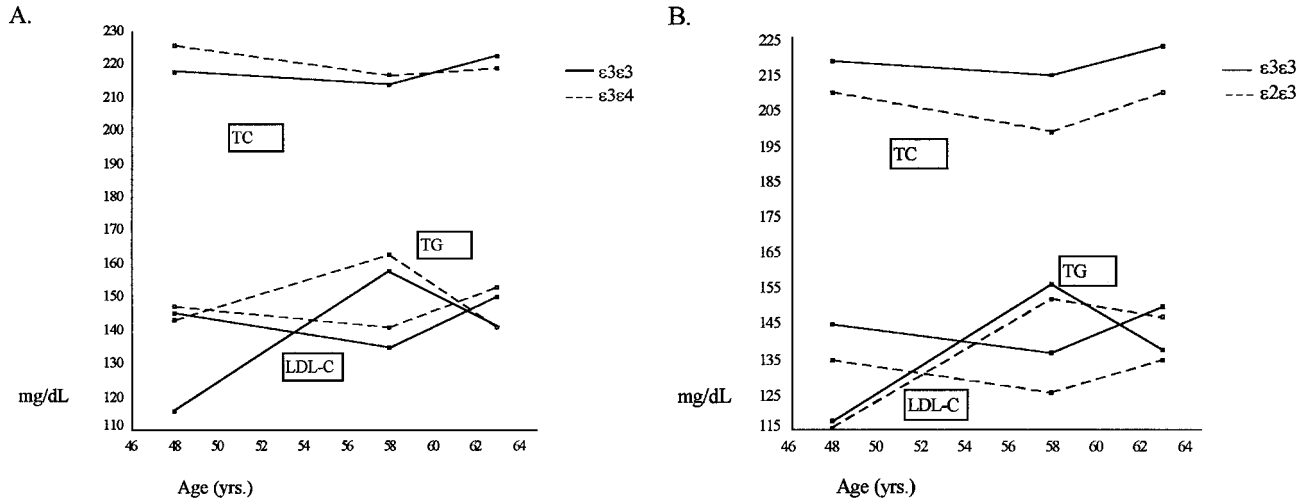

Figure 1 TC, LDL-C, and TG levels, by APOE genotype, in unrelated subjects, at exams 1, 2, and 3; mean ages were 48, 58, and 63 years, respectively. An APOE ε3ε3 group is contrasted with the ε3ε4 group (*A*) and with the ε2ε3 group (*B*).

There are at least three possible explanations for the differences in changes in lipid levels, over time, between ε3ε3 and ε3ε4 APOE genotypes: (1) ascertainment bias, (2) genotype-specific response to a general change in the environment over time, or (3) true aging effects, in which the change in lipids with increasing age varies with genotype. Ascertainment bias is unlikely, since these men were selected on the basis of twin status, which is not expected to correlate with lipid levels. The men did have to be present at the third exam (mean age 63 years) in order to be genotyped; however, their lipid levels at each age were typical of those seen in the general population (Jarvik et al. 1994), and their APOE genotypes also were representative of populations described elsewhere (Sing and Davignon 1985). The results cannot be due to differential survival within the sample, since the analysis considers only men present at all exams. APOE genotype–dependent change in lipids in response to dietary and weight changes have been described in some populations (Gueguen et al. 1989; Lehtimaki et al. 1992; Uusitupa et al. 1992; Jenkins et al. 1993; Lopez Miranda et al. 1994). If subjects significantly changed their diet over the course of the study, their responses could be genotype dependent. However, it is unlikely that the TG decline in the ε3ε4 group was due to a reduction in dietary fat or cholesterol, since LDL-C did not decline. Additionally, adjustment of the lipid levels for BMI at each exam did not change the results. A true aging effect is the most likely explanation for this result. Lipid levels increase with age until the 7th decade and then decline in males (National and Health 1980; Hershcopf et al. 1982; Alvarez et al. 1984; Newschaffer et al. 1992). This decline in lipid levels in longitudinal studies of males after the age of 60 years has been shown to be due in part to an aging effect that is not due to changes in weight, diet, or exercise (Hershcopf et al. 1982). It is possible that the mechanism responsible for these aging effects in lipids is sensitive to the differences in the APOE ε3 product versus the ε4-gene product.

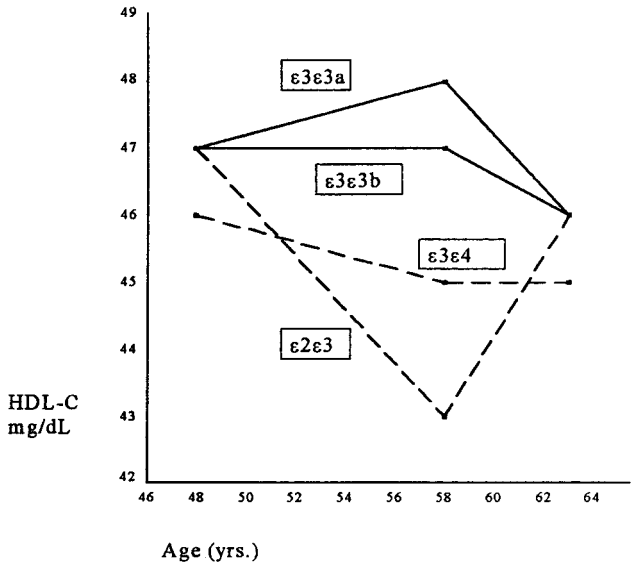

Figure 2 HDL-C levels, by APOE genotype, in unrelated subjects, at exams 1, 2, and 3; mean ages were 48, 58, and 63 years, respectively. The APOE ε3ε3a group is contrasted with the ε2ε3 group, and the ε3ε3b group is contrasted with the ε3ε4 group.

The lack of an exam–by–ε3ε3-versus-ε2ε3-genotype interaction in the prediction of TC levels in this longitudinal study contrasts with a cross-sectional study (Reilly et al. 1992) that suggested that the regression of TC on age differs between ε2ε3-genotype and ε3ε3-genotype

males of age 26–63 years (mean 44 years); however, differences in either the age distributions of the samples or the designs of the studies may account for the discordant results. In a cross-sectional study, APOE-genotype effects on plasma apoE concentration were found to differ with age in both males and females, with the variance due to APOE genotype decreasing in the older ages (Zerba et al. 1996), suggesting that the APOE-genotype effects on plasma level of apoE protein diminishes with age.

Fifty-five to 60% of the population is expected to have the ε3ε3 genotype, 23%–25% the ε3ε4 genotype, 12%–14% the ε2ε3 genotype, 2%–3% the ε4ε4 genotype, 2% the ε2ε4 genotype, and <1% the ε2ε2 genotype. Thus, genetic variation at this locus is quite common and is expected to contribute significantly to population variation in lipid levels. Changes in the APOE-genotype effects with age may result in difficulties both in comparing studies in different age groups and in interpreting studies in which age is not carefully considered. It is possible that such confounding effects may in part be responsible for the conflicting results found by various studies examining the effects of (*a*) APOE genotype on prediction of CAD (Menzel et al. 1983; Cumming and Robertson 1984); reviewed by (Davignon et al. 1988) and (*b*) the magnitude of APOE-genotype effects on lipids and lipoproteins (Sing and Davignon 1985; Davignon et al. 1987). This study addresses only the 15-year window of mean age 48–63 years. For LDL-C and TC, the data suggest that equalization of genotype means between ε3ε3-genotype and ε3ε4-genotype males may begin to occur at age <48 years. Given the documented decline in the frequency of the ε4 allele with age, in addition to the changes in genotype effects with age that have been described here, possible age interactions should be carefully considered in studies of APOE-genotype effects on determinants of health in aging populations.

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